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Effects of Leptin and Insulin on CA III Expression in Rat Adipose Tissue

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Studies on the biochemical and molecular mechanisms underlying obesity have shown that the expression of some proteins was decreased with obesity in rat adipose tissue. One of these proteins is carbonic anhydrase III (CA III) which constitutes 24% of the cytosolic protein content and its function is unclear. A freshly isolated rat adipose cell culture model was used to examine the effect of leptin and insulin on CA III expression. It was found that leptin decreased CA III expression while insulin increased it which suggests that the decrease in CA III expression observed in obesity in rat adipose tissue may be related to hyperleptinemia.

Keywords: Leptin; Insulin; Carbonic anhydrase III; Reverse transcriptase–PCR; Rat adipose tissue

INTRODUCTION

Carbonic anhydrases (CA; carbonate hydrolyase, EC 4.2.1.1) are zinc-containing enzymes, which reversibly catalyse the hydration of carbon dioxide to bicarbonate and hydrogen ions.[1] Fourteen CA isoenzymes with different tissue distribution and kinetic properties have been described up to the present. These isoenzymes have been shown to be involved in important physiological processes such as pH regulation, transport of CO_2/HCO_3^- between tissues and lung, electrolyte secretion in some tissues, and metabolic pathways such as gluconeogenesis, lipogenesis and ureagenesis.[2]

CA III which is known as muscle CA has unusual kinetic properties such as low activity and resistance to sulphonamides.[3] In mammals, its major site of expression is skeletal muscle, where it represents approximately 8–15% of the soluble protein of slow twitch (type I fiber) red skeletal muscle.[4,5] Several researches have shown that the tissue with highest CA III abundance is the white adipose tissue of the rat with a concentration of 24% of the cytosolic protein.[4,6] Although, its main function in rat adipose tissue is unclear, it has been supposed that CA III provides bicarbonate ion necessary to convert acetyl–CoA into malonyl–CoA, the building block of fatty acids.[6] Its amount and activity in rat adipose tissue decreases with obesity.[3,6,7] The reason for this is not known but it has been suggested that CA III expression is directly or indirectly regulated by high insulin levels observed in obesity which change adipose tissue metabolism.[3] In addition, Dodgson *et al.* reported that insulin decreased CA III concentration in 3T3 adipocytes while CA II was unaffected.[8]

Leptin, the *ob* gene product, is primarily secreted from adipose tissue and acts on the hypothalamus as a signal for regulating food intake and energy expenditure. The rate of leptin secretion and its plasma concentration which is controlled by environmental and hormonal factors, are related to total fatty mass in the human and rodent.[9] The existence of leptin receptors in peripheral tissues such as adipose tissue, skeletal muscle, lung and liver shows that this hormone has autocrine and paracrine effects on these tissues besides its systemic effects.[10] Leptin inhibits fatty acid and triglyceride synthesis and increases lipid oxidation in adipose tissue.[11] This effect of leptin is performed by changing the levels of acetyl CoA carboxylase which is the key enzyme in fatty acid synthesis.[10]

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In this study, we have investigated the effect of leptin and insulin on CA III expression in rat epididymal adipose tissue. This investigation shows that leptin decreases CA III expression leading to the conclusion that the CA III decrease observed in obese rats may be attributed to leptin.

MATERIALS AND METHOD

Adipocyte Isolation and Tissue Culture

Following euthanasia by ether anesthesia at between 9 and 10 a.m. adipose tissue was taken from the epididymal fat pads of five Sprague–Dawley rats (150–250 g). The total tissue was broken into 2–4 mm^3 pieces and visible vessels, blood clots and genital organ pieces were removed. Minced epididymal fat tissue was digested at 37°C for 2–3 h in a buffer containing type II collagenase (1 mg/mL), albumin (3.5%), and glucose (0.55 mM). Cells were then washed three times with Krebs–Ringer bicarbonate buffer (pH 7.4) and cultured in a sterile Iscove's modified Dulbecco's medium (IMDM) containing 25 mM HEPES, pH 7.2, 25 mM glucose. 100 IU/mL penicillin, 100 μg/mL streptomycin and 10% fetal calf serum. After 72 h the cell culture medium was changed with the same medium. Four groups were setup and three groups of these were formed by addition of 1) 25 ng/mL recombinant rat leptin, 2) 100 nM insulin, 3) 25 ng/mL recombinant rat leptin and 100 nM insulin. The fourth group was the control group. The samples were then incubated at 37°C under 5% CO_2 for 24 h.

Reverse Transcriptase PCR

mRNA was semiquantified by reverse transcriptase PCR. Total RNA was obtained from adipocytes using Nucleospin RNA II kit (Macherey–Nagel). Reverse transcription PCR was done using Superscript One Step RT–PCR kit (Life Technology). Primers for CA III were sense 5′-CGCTGTGGTTGGCATTTTTC-3′ and antisense 5′-AGGCTGCGCACGTTGGCCAT-3′ and β- actin, used as a standard housekeeping gene, sense 5′-TTGTAACCAACTGGGACGATATGG-3′ antisense 5′-GATCTTGATCTTCATGGTGCTAGG-3′. RT–PCR conditions were as follows: 60 min at 45°C for reverse transcription and 15 min at 70°C for this process to be terminated. Then, denaturation for 30s at 94°C, annealing for 60 s at 53°C, and elongation for 1 min 72°C with 35 cycles.[12] The PCR products were subjected to electrophoresis on 2% agarose jel containing ethidium bromide and the fluorescent intensity of each band was measured with an image analyser (BioDocAnalyze: Biometra) the obtained results were expressed as the ratio of the fluorescent density of CA III/β-actin electrophoretic bands.

RESULTS AND DISCUSSION

A number of studies have been done on animals to elucidate the molecular and biochemical mechanisms

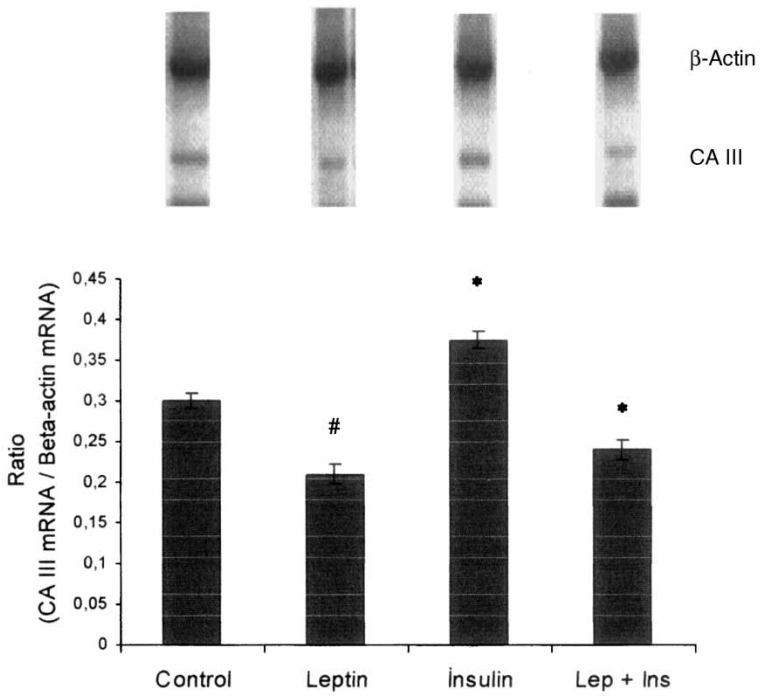

FIGURE 1 The effect of leptin (25 ng/ml) and insulin (100 nm) on CA III expression in rat epididymal adipose tissue. #, ($p < 0{,}001$), *, ($p < 0.05$) compared with control group (the paired Student's *t*-test) (n = 6).

underlying obesity. These studies have described some functional and molecular differences between adipose tissue isolated from lean and obese individuals.[6,7] For example, obese animals have increased insulin resistance and rate of fatty acid synthesis. In addition, the expression of some enzymes such as ATP–citrate lyase and acetyl–CoA carboxylase were increased, while some other proteins were decreased. One of these proteins is CA III.[3,6,7]

The levels of hormones such as insulin and leptin change in blood with obesity and this causes important changes in the activities of key enzymes which have roles in fuel metabolism.[10] Therefore, in our experiments hyper doses of these hormones were used.

We observed that insulin increased CA III expression in rat epididymal adipose tissue (Figure 1). In previous studies, the possible role of insulin in the obesity-dependent loss of adipose tissue CA III was examined and it was reported that insulin might play an important role in this process.[3,8] However, in those researches, the direct effect of insulin on CA III expression was not investigated. Since insulin increases *de novo* fatty acid synthesis in adipose tissue and it is known that the function of CA III is to provide bicarbonate ion for this pathway, the increased expression of CA III in rat adipose tissue is reasonable.

It was found that leptin decreased CA III expression in rat epididymal adipose tissue (Figure 1). Leptin has been shown to mediate fatty acid metabolism by changing mRNA levels and concentrations of the key enzymes.[10] Since leptin has lipolytic effects, lipogenesis should be decreased during its effect.[11] This might be accomplished by a decrease in CA III expression in rat adipose tissue. The addition of insulin and leptin to cultured adipocytes caused a significant decrease in CA III expression. This may have resulted from the antagonist effect of leptin on the action of insulin on target tissues as it has been observed in many studies.[13]

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